

"Combination Therapy"

Field of the Invention

This application relates to combination therapy and its use in methods of treatment. In particular, it relates to the treatment of cancer cells comprising a p53 mutation with a death receptor ligand, e.g. a FAS (CD95 or TNF receptor 2) receptor ligand, and a chemotherapeutic agent.

Background to the Invention

Breast, oesophageal, colorectal, all forms of GI cancer and head and neck cancers are highly malignant with overall 5-year survival rates of less than 50%. The clinical outcome of these patients is predetermined by the presence of widely disseminated tumour cells termed micrometastases with potential for metastatic growth, prior to clinical presentation. Approximately 50% of oesophageal cancer patients are selected for surgical therapy

with 30% 5-year survival for this patient sub-group. Randomised clinical trials of neoadjuvant 5FU-based chemotherapy combined with fractionated radiotherapy have demonstrated improvements in survival of 10-20%, although the overall 5-year outcome for the treated groups remains at 30-35%. Those patients who demonstrate complete pathological response in their primary tumours as a result of neoadjuvant treatment have a five-year survival of 80%. Conversely, those patients who do not respond to 5FU-based chemotherapy are denied the opportunity for earlier treatment by surgery or a different neoadjuvant chemotherapeutic based regimen.

Colorectal cancer (CRC) is the second highest cause of cancer mortality in the western world. Approximately 40-50% of colon cancers will harbour mutations in the tumour suppressor gene p53. There is increasing evidence that not all p53 mutations result in absolute loss of function. Functional activities or properties of mutant proteins include retained wild-type activity [49], loss of function [50], gain of function [51, 52], dominant-negative effect [53] and temperature sensitivity. Two of the most prevalent p53 mutations in colon cancer occur at the codon 'hotspots' 175 and 248. These missense mutations result in the substitution of either histidine (R175H mutation) or tryptophan (R248W mutation) for arginine.

The most frequently used chemotherapeutic agents for the treatment of colorectal cancers are the

fluoropyrimidine 5-fluorouracil (5-FU), the topoisomerase-I inhibitor Irinotecan (CPT-11) and the platinum agent Oxaliplatin. The thymidylate synthase inhibitor Tomudex (TDX) is also still used in the treatment of advanced colorectal cancer. 5-FU acts primarily by inhibiting the enzyme thymidylate synthase (TS) [40]. Because TS is a key enzyme in the *de novo* synthesis of thymidylate, its inhibition results in imbalances in intracellular dNTP pools and inhibition of DNA synthesis [41]. 5-FU also has direct effects on DNA and RNA, which contributes to its cytotoxicity [42]. CPT-11 is a prodrug that is hydrolysed to its active metabolite SN-38 by carboxylesterases [43]. It exerts its cytotoxic effect through the inhibition of topoisomerase-I [44]. Topo-I inhibitors stabilise the complex between topo-I and DNA which collide with moving DNA replication forks, leading to double stranded DNA breaks. Oxaliplatin is a third generation platinum cytotoxic in which a diaminocyclohexane (DACH) moiety replaces the amine groups present in cisplatin [44]. Although, like cisplatin, oxaliplatin also causes DNA-platinum adducts, it forms less of these than cisplatin and yet demonstrates more cytotoxicity. It is suggested that the oxaliplatin-DNA adducts are more lethal than cisplatin adducts [45]. Tomudex is a specific TS inhibitor. It is transported into cells via a reduced folate carrier and then undergoes extensive polyglutamation. The polyglutamated forms are up to 100 times more active than the parent compound [46]. 5-FU alone is used extensively as adjuvant

chemotherapy in patients with early stage CRC [47]. Combinations of 5-FU together with either CPT-11 or Oxaliplatin are the standard of care for patients with advanced CRC [48].

Nevertheless, despite improvements in the efficacy of chemotherapy drugs used in the treatment of colorectal cancer, response rates are of the order of 45-50% for the most effective drug combinations.

The Fas/CD95 receptor is a 48 kDa member of the tumour necrosis factor receptor (TNFR) family [36]. The signalling members of the TNFR superfamily can be divided into two groups based on the composition of their cytoplasmic region. The death receptors (Fas/CD95 together with the receptors TNFR1, TNFR2, DR4 and DR5) contain a death domain in the cytoplasmic part of the receptor while the other group does not. This death domain is essential for transduction of the apoptotic signal. Binding of the Fas death receptor to its cognate ligand, called FasL, results in recruitment of FADD and caspase 8 to the receptor, and the formation of the death-inducing signalling complex (DISC) [17]. Active caspase 8 in turn activates downstream executioner caspases including caspase 3, which cleave a cassette of proteins resulting in cell death [37]. Caspase 8 also activates the mitochondrial cell death pathway through cleavage of the protein Bid. A variety of chemotherapeutic agents have been shown to cause up-regulation of the Fas/CD95 receptor in cancer cell lines. Fas/CD95 induction has also been

documented following treatment of cancer cell lines with UV radiation [38]. The ability of chemotherapy drugs to induce the receptor has stimulated interest in targeting the Fas/CD95 death receptor with either therapeutic antibodies or peptides to enhance cell kill.

Thus, there is an urgent need for improved therapeutic strategies.

Summary of the Invention

As described herein, the present inventors have shown that by combining treatment using a death receptor ligand, such as an anti FAS antibody, with a thymidylate synthase inhibitor such as 5-FU, a topoisomerase inhibitor such as CPT-11, an antifolate drug, such as raltitrexed (RTX) or pemetrexed (MTA, Alimta), a platinum based cytotoxic such as oxaliplatin, a synergistic effect is achieved in the killing of cancer cells. However, the inventors have further shown that, for some chemotherapeutic agents, such as the platinum based cytotoxics, the synergistic cytotoxic effect is p53 dependent. As described in the Examples, the synergy observed for the combinations comprising such chemotherapeutic agents was not observed for corresponding p53 mutant cells. However, to the inventors' surprise, it was demonstrated that the synergistic cytotoxic properties obtained using the combination of death receptor ligand with a

chemotherapeutic agent was maintained for certain chemotherapeutic agents, such as RTX and CPT-11.

Accordingly, in a first aspect, the present invention provides a method of killing cancer cells having a p53 mutation, said method comprising the separate, sequential or simultaneous administration to said cells of a therapeutically effective amount of a) a specific binding member which binds to a cell death receptor or a nucleic acid encoding said binding member and (b) a chemotherapeutic agent, wherein the chemotherapeutic agent is a topoisomerase inhibitor or a thymidylate synthase inhibitor.

In a second aspect, the present invention provides a method of treating cancer cells having a p53 mutation comprising the separate, sequential or simultaneous administration to a mammal in need thereof of a therapeutically effective amount of a) a specific binding member which binds to a cell death receptor or a nucleic acid encoding said binding member and (b) a chemotherapeutic agent, wherein the chemotherapeutic agent is a topoisomerase inhibitor or a thymidylate synthase inhibitor.

The specific binding member and the chemotherapeutic agent may be administered simultaneously, sequentially or simultaneously. In preferred embodiments of the invention, the chemotherapeutic agent is administered prior to the specific binding

member.

In a third aspect, there is provided the use of
(a) a specific binding member which binds to a cell death receptor or a nucleic acid encoding said binding member and

(b) a chemotherapeutic agent, wherein the chemotherapeutic agent is a topoisomerase inhibitor or a thymidylate synthase inhibitor in the preparation of a medicament for treating cancer, wherein the cancer cells comprise a p53 mutation.

In a fourth aspect, there is provided a product comprising a) a specific binding member which binds to a cell death receptor or a nucleic acid encoding said binding member and (b) a chemotherapeutic agent as a combined preparation for the simultaneous, separate or sequential use in the treatment of cancer, wherein the chemotherapeutic agent is a topoisomerase inhibitor or a thymidylate synthase inhibitor, and wherein the cancer cells comprise a p53 mutation.

According to a fifth aspect, there is provided a pharmaceutical composition for the treatment of a cancer characterised by the presence of a p53 mutation, wherein the composition comprises a) a specific binding member which binds to a cell death receptor or a nucleic acid encoding said binding member and (b) a chemotherapeutic agent, wherein the chemotherapeutic agent is a topoisomerase inhibitor

or a thymidylate synthase inhibitor and (c) a pharmaceutically acceptable excipient, diluent or carrier.

In a sixth aspect, there is provided a kit for the treatment of a cancer characterised by the presence of a p53 mutation, said kit comprising a) a specific binding member which binds to a cell death receptor or a nucleic acid encoding said binding member and (b) a chemotherapeutic agent, wherein the chemotherapeutic agent is a topoisomerase inhibitor or a thymidylate synthase inhibitor and (c) instructions for the administration of (a) and (b) separately, sequentially or simultaneously.

Preferred thymidylate synthase inhibitors for use in the invention are antifolate thymidylate synthase inhibitors, such as raltrexed (TDX) or pemetrexed (MTA). Preferred topoisomerase inhibitors for use in the invention are topoisomerase I inhibitors, such as camptothecins, such as CPT-11.

In a preferred embodiment of the invention, the chemotherapeutic agent is an antifolate, such as raltrexed (TDX) or pemetrexed (MTA) or a topoisomerase-I inhibitor, such as CPT-11 or Particularly preferred examples of antifolates and topoisomerase-I inhibitors for use in the invention are TDX and irinotecan (CPT-11). Unless, the context demand otherwise, reference to CPT-11 should be taken to encompass CPT-11 or its active metabolite SN-38.

The invention may be used to treat any cancer comprising cells having a p53 mutation. The mutation may partially or totally inactivate p53 in a cell. In one embodiment of the invention, the p53 mutation is a p53 mutation, which totally inactivates p53. In another embodiment, the p53 mutation is a missense mutation resulting in the substitution of histidine (R175H mutation). In another embodiment, the p53 mutation is a missense mutation resulting in the substitution of tryptophan (R248W mutation) for arginine.

In preferred embodiments of the invention, the cancer is one or more of colorectal, breast, ovarian, cervical, gastric, lung, liver, skin and myeloid (e.g. bone marrow) cancer. In a particular embodiment of the invention, the cancer is a colorectal cancer.

The binding member for use in the invention may bind to any death receptor. Death receptors include, Fas, TNFR, DR-3, DR-4 and DR-5. In preferred embodiments of the invention, the death receptor is FAS.

In preferred embodiments of the invention, the binding member is an antibody or a fragment thereof.

In particularly preferred embodiments, the binding member is the FAS antibody CH11 (Yonehara, S., Ishii, A. and Yonehara, M. (1989) J. Exp. Med. 169, 1747-1756) (available commercially e.g. from Upstate Biotechnology, Lake Placid, NY).

In preferred embodiments, the binding member comprises at least one human constant region.

The concentrations of binding members and chemotherapeutic agents used are preferably sufficient to provide a synergistic effect. Synergism is preferably defined as an RI of greater than unity using the method of Kern as modified by Romanelli (13, 14). The RI may be calculated as the ratio of expected cell survival (S_{exp} , defined as the product of the survival observed with drug A alone and the survival observed with drug B alone) to the observed cell survival (S_{obs}) for the combination of A and B ($RI = S_{exp}/S_{obs}$). Synergism may then be defined as an RI of greater than unity.

In preferred embodiments of the invention, said specific binding member and chemotherapeutic agent are provided in concentrations sufficient to produce an RI of greater than 1.5, more preferably greater than 2.0, most preferably greater than 2.25.

The combined medicament thus preferably produces a synergistic effect when used to treat tumour cells having a p53 mutant genotype.

A seventh aspect of the present invention therefore provides a medicament for use in treating p53 mutant tumour cells, the medicament comprising at least one antibody directed at FAS receptor and at least one cancer chemotherapeutic agent, wherein the

chemotherapeutic agent is a topoisomerase inhibitor or a thymidylate synthase inhibitor.

Preferred features of each aspect of the invention are as for each of the other aspects mutatis mutandis.

Detailed Description

Binding members

In the context of the present invention, a "binding member" is a molecule which has binding specificity for another molecule, in particular a receptor, in particular a death receptor. A binding member of the invention and for use in the invention may be any moiety, for example an antibody or ligand, which can bind to a death receptor.

Antibodies

An "antibody" is an immunoglobulin, whether natural or partly or wholly synthetically produced. The term also covers any polypeptide, protein or peptide having a binding domain which is, or is homologous to, an antibody binding domain. These can be derived from natural sources, or they may be partly or wholly synthetically produced. Examples of antibodies are the immunoglobulin isotypes and their isotypic subclasses and fragments which comprise an antigen binding domain such as Fab, scFv, Fv, dAb, Fd; and diabodies.

The binding member of the invention may be an antibody such as a monoclonal or polyclonal antibody, or a fragment thereof. The constant region of the antibody may be of any class including, but not limited to, human classes IgG, IgA, IgM, IgD and IgE. The antibody may belong to any sub class e.g. IgG1, IgG2, IgG3 and IgG4. IgG1 is preferred.

As antibodies can be modified in a number of ways, the term "antibody" should be construed as covering any binding member or substance having a binding domain with the required specificity. Thus, this term covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including any polypeptide comprising an immunoglobulin binding domain, whether natural or wholly or partially synthetic. Chimeric molecules comprising an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023.

It has been shown that fragments of a whole antibody can perform the function of binding antigens. Examples of such binding fragments are (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward, E.S. et al., Nature 341:544-546

(1989)) which consists of a VH domain; (v) isolated CDR regions; (vi) F(ab')₂ fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al., Science 242:423-426 (1988); Huston et al., PNAS USA 85:5879-5883 (1988)); (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; P. Hollinger et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993)).

A fragment of an antibody or of a polypeptide for use in the present invention generally means a stretch of amino acid residues of at least 5 to 7 contiguous amino acids, often at least about 7 to 9 contiguous amino acids, typically at least about 9 to 13 contiguous amino acids, more preferably at least about 20 to 30 or more contiguous amino acids and most preferably at least about 30 to 40 or more consecutive amino acids.

A "derivative" of such an antibody or polypeptide, or of a fragment antibody means an antibody or polypeptide modified by varying the amino acid sequence of the protein, e.g. by manipulation of the nucleic acid encoding the protein or by altering the protein itself. Such derivatives of the natural amino acid sequence may involve insertion, addition, deletion and/or substitution of one or more amino

acids, preferably while providing a peptide having death receptor, e.g. FAS neutralisation and/or binding activity. Preferably such derivatives involve the insertion, addition, deletion and/or substitution of 25 or fewer amino acids, more preferably of 15 or fewer, even more preferably of 10 or fewer, more preferably still of 4 or fewer and most preferably of 1 or 2 amino acids only.

The term "antibody" includes antibodies which have been "humanised". Methods for making humanised antibodies are known in the art. Methods are described, for example, in Winter, U.S. Patent No. 5,225,539. A humanised antibody may be a modified antibody having the hypervariable region of a monoclonal antibody and the constant region of a human antibody. Thus the binding member may comprise a human constant region.

The variable region other than the hypervariable region may also be derived from the variable region of a human antibody and/or may also be derived from a monoclonal antibody. In such case, the entire variable region may be derived from murine monoclonal antibody and the antibody is said to be chimerised. Methods for making chimerised antibodies are known in the art. Such methods include, for example, those described in U.S. patents by Boss (Celltech) and by Cabilly (Genentech). See U.S. Patent Nos. 4,816,397 and 4,816,567, respectively.

It is possible to take monoclonal and other antibodies and use techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementary determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP-A-184187, GB 2188638A or EP-A-239400. A hybridoma or other cell producing an antibody may be subject to genetic mutation or other changes, which may or may not alter the binding specificity of antibodies produced.

A typical antibody for use in the present invention is a humanised equivalent of CH11 or any chimerised equivalent of an antibody that can bind to the FAS receptor and any alternative antibodies directed at the FAS receptor that have been chimerised and can be use in the treatment of humans. Furthermore, the typical antibody is any antibody that can cross-react with the extracellular portion of the FAS receptor and either bind with high affinity to the FAS receptor, be internalised with the FAS receptor or trigger signalling through the FAS receptor.

Production of Binding Members

The binding members for use in the present invention may be generated wholly or partly by chemical

synthesis. The binding members can be readily prepared according to well-established, standard liquid or, preferably, solid-phase peptide synthesis methods, general descriptions of which are broadly available (see, for example, in J.M. Stewart and J.D. Young, Solid Phase Peptide Synthesis, 2nd edition, Pierce Chemical Company, Rockford, Illinois (1984), in M. Bodanzsky and A. Bodanzsky, The Practice of Peptide Synthesis, Springer Verlag, New York (1984); and Applied Biosystems 430A Users Manual, ABI Inc., Foster City, California), or they may be prepared in solution, by the liquid phase method or by any combination of solid-phase, liquid phase and solution chemistry, e.g. by first completing the respective peptide portion and then, if desired and appropriate, after removal of any protecting groups being present, by introduction of the residue X by reaction of the respective carbonic or sulfonic acid or a reactive derivative thereof.

Another convenient way of producing a binding member suitable for use in the present invention is to express nucleic acid encoding it, by use of nucleic acid in an expression system. Thus the present invention further provides the use of (a) nucleic acid encoding a specific binding member which binds to a cell death receptor and (b) a chemotherapeutic agent in the preparation of a medicament for treating cancer.

Nucleic acid for use in accordance with the present invention may comprise DNA or RNA and may be wholly

or partially synthetic. In a preferred aspect, nucleic acid for use in the invention codes for a binding member of the invention as defined above. The skilled person will be able to determine substitutions, deletions and/or additions to such nucleic acids which will still provide a binding member suitable for use in the present invention.

Nucleic acid sequences encoding a binding member for use with the present invention can be readily prepared by the skilled person using the information and references contained herein and techniques known in the art (for example, see Sambrook, Fritsch and Maniatis, "Molecular Cloning", A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989, and Ausubel et al, Short Protocols in Molecular Biology, John Wiley and Sons, 1992), given the nucleic acid sequences and clones available. These techniques include (i) the use of the polymerase chain reaction (PCR) to amplify samples of such nucleic acid, e.g. from genomic sources, (ii) chemical synthesis, or (iii) preparing cDNA sequences. DNA encoding antibody fragments may be generated and used in any suitable way known to those of skill in the art, including by taking encoding DNA, identifying suitable restriction enzyme recognition sites either side of the portion to be expressed, and cutting out said portion from the DNA. The portion may then be operably linked to a suitable promoter in a standard commercially available expression system. Another recombinant approach is to amplify the relevant portion of the DNA with suitable PCR primers.

Modifications to the sequences can be made, e.g. using site directed mutagenesis, to lead to the expression of modified peptide or to take account of codon preferences in the host cells used to express the nucleic acid.

The nucleic acid may be comprised as construct(s) in the form of a plasmid, vector, transcription or expression cassette which comprises at least one nucleic acid as described above. The construct may be comprised within a recombinant host cell which comprises one or more constructs as above. Expression may conveniently be achieved by culturing under appropriate conditions recombinant host cells containing the nucleic acid. Following production by expression a specific binding member may be isolated and/or purified using any suitable technique, then used as appropriate.

Binding members-encoding nucleic acid molecules and vectors for use in accordance with the present invention may be provided isolated and/or purified, e.g. from their natural environment, in substantially pure or homogeneous form, or, in the case of nucleic acid, free or substantially free of nucleic acid or genes origin other than the sequence encoding a polypeptide with the required function.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, yeast and baculovirus systems. Mammalian

cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells, NSO mouse melanoma cells and many others. A common, preferred bacterial host is *E. coli*.

The expression of antibodies and antibody fragments in prokaryotic cells such as *E. coli* is well established in the art. For a review, see for example Plückthun, *Bio/Technology* 9:545-551 (1991). Expression in eukaryotic cells in culture is also available to those skilled in the art as an option for production of a binding member, see for recent review, for example Reff, *Curr. Opinion Biotech.* 4:573-576 (1993); Trill et al., *Curr. Opinion Biotech.* 6:553-560 (1995).

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. phage, or phagemid, as appropriate. For further details see, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual: 2nd Edition*, Cold Spring Harbor Laboratory Press (1989). Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in Ausubel et al. eds.,

Short Protocols in Molecular Biology, 2nd Edition,
John Wiley & Sons (1992).

The nucleic acid may be introduced into a host cell by any suitable means. The introduction may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage.

Marker genes such as antibiotic resistance or sensitivity genes may be used in identifying clones containing nucleic acid of interest, as is well known in the art.

The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells under conditions for expression of the gene.

The nucleic acid may be integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome in accordance with standard techniques. The nucleic acid may be on an extra-chromosomal vector within the cell, or

otherwise identifiably heterologous or foreign to the cell.

Chemotherapeutic Agents

As described above, the present invention is based on the surprising demonstration that, contrary to the synergism demonstrated for antineoplastic combination therapies such as CH-11 and cisplatin, which is p53 dependent, the synergistic cytotoxic effects of combination therapies comprising a death receptor ligand and a topoisomerase inhibitor or a thymidylate synthase inhibitor is p53 independent.

Accordingly, the invention provides novel effective drug combinations for the treatment of p53 mutant tumours.

Any suitable a topoisomerase inhibitor or thymidylate synthase inhibitor may be used in the invention.

Examples of thymidylate synthase inhibitor antifolates include fluoropyrimidines such as 5-FU and antifolates such as RTX(TDX) and MTA. Examples of topoisomerase inhibitors include topoisomerase-I inhibitors, such as camptothecins and topoisomerase-II inhibitors.

Preferred topoisomerase inhibitors or thymidylate synthase inhibitors for use in the invention are those agents which demonstrate synergistic cytotoxic

properties in combination with death receptor ligands such as CH-11 on p53 mutant cells, for example p53 null cells, preferably with an RI of greater than 1.5, preferably greater than 2.0.

In one particularly preferred embodiment, the agent is CPT-11.

In another particularly preferred embodiment, the agent is TDX.

Treatment

"Treatment" includes any regime that can benefit a human or non-human animal. The treatment may be in respect of an existing condition or may be prophylactic (preventative treatment). Treatment may include curative, alleviation or prophylactic effects.

"Treatment of cancer" includes treatment of conditions caused by cancerous growth and includes the treatment of neoplastic growths or tumours. Examples of tumours that can be treated using the invention are, for instance, sarcomas, including osteogenic and soft tissue sarcomas, carcinomas, e.g., breast-, lung-, bladder-, thyroid-, prostate-, colon-, rectum-, pancreas-, stomach-, liver-, uterine-, cervical and ovarian carcinoma, lymphomas, including Hodgkin and non-Hodgkin lymphomas, neuroblastoma, melanoma, myeloma, Wilms tumor, and leukemias, including acute lymphoblastic leukaemia

and acute myeloblastic leukaemia, gliomas and retinoblastomas.

The compositions and methods of the invention may be particularly useful in the treatment of existing cancer and in the prevention of the recurrence of cancer after initial treatment or surgery.

Administration

Binding members and chemotherapeutic agents may be administered simultaneously, separately or sequentially.

Where administered separately or sequentially, they may be administered within any suitable time period e.g. within 1, 2, 3, 6, 12, 24, 48 or 72 hours of each other. In preferred embodiments, they are administered within 6, preferably within 2, more preferably within 1, most preferably within 20 minutes of each other.

In a preferred embodiment, they are administered as a pharmaceutical composition, which will generally comprise a suitable pharmaceutical excipient, diluent or carrier selected dependent on the intended route of administration.

Binding members and chemotherapeutic agents of and for use in the present invention may be administered to a patient in need of treatment via any suitable route. The precise dose will depend upon a number of

factors, including the precise nature of the member (e.g. whole antibody, fragment or diabody) and chemotherapeutic agent.

Some suitable routes of administration include (but are not limited to) oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. Intravenous administration is preferred.

It is envisaged that injections (intravenous) will be the primary route for therapeutic administration of compositions although delivery through a catheter or other surgical tubing is also envisaged. Liquid formulations may be utilised after reconstitution from powder formulations.

For intravenous, injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

4,485,045 and 4,544,545. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. % cholesterol, the selected proportion being adjusted for the optimal rate of the polypeptide leakage.

Examples of the techniques and protocols mentioned above and other techniques and protocols which may be used in accordance with the invention can be found in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A. (ed), 1980.

The binding member, agent, product or composition may be administered in a localised manner to a tumour site or other desired site or may be delivered in a manner in which it targets tumour or other cells. Targeting therapies may be used to deliver the active agents more specifically to certain types of cell, by the use of targeting systems such as antibody or cell specific ligands. Targeting may be desirable for a variety of reasons, for example if the agent is unacceptably toxic, or if it would otherwise require too high a dosage, or if it would not otherwise be able to enter the target cells.

Pharmaceutical Compositions

As described above, the present invention extends to pharmaceutical composition for the treatment of a cancer characterised by the presence of a p53

mutation, wherein the composition comprises a) a specific binding member which binds to a cell death receptor or a nucleic acid encoding said binding member and (b) a chemotherapeutic agent, wherein the chemotherapeutic agent is a thymidylate synthase inhibitor, a topoisomerase-I inhibitor or a fluoropyrimidine. Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention may comprise, in addition to active ingredients, a pharmaceutically acceptable excipient, carrier, buffer stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. intravenous.

The formulation may be a liquid, for example, a physiologic salt solution containing non-phosphate buffer at pH 6.8-7.6, or a lyophilised powder.

Dose

The binding members, agents, products or compositions are preferably administered to an individual in a "therapeutically effective amount", this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated.

As described herein, the concentrations are preferably sufficient to show a synergistic effect. Prescription of treatment, e.g. decisions on dosage etc, is ultimately within the responsibility and at the discretion of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners.

The optimal dose can be determined by physicians based on a number of parameters including, for example, age, sex, weight, severity of the condition being treated, the active ingredient being administered and the route of administration. For example, with respect to binding members, in general, a serum concentration of polypeptides and antibodies that permits saturation of receptors is desirable. A concentration in excess of approximately 0.1nM is normally sufficient. For example, a dose of 100mg/m² of antibody provides a serum concentration of approximately 20nM for approximately eight days.

As a rough guideline, doses of antibodies may be given in amounts of 1ng/kg- 500mg/kg of patient weight. Equivalent doses of antibody fragments should be used at the same or more frequent intervals in order to maintain a serum level in excess of the concentration that permits saturation of death receptor.

Doses of the binding members may be given at any suitable dose interval e.g. daily, once, twice or thrice weekly.

For example, the periods of administration of a humanised antibody could be from 1 bolus injection to weekly administration for up to one year in combination with chemotherapeutic agents. The likely dose is upwards of 1mg/per kg/per patient.

Doses of chemotherapeutic agent will depend on the factors described above but preferably are administered in doses which are within the normal range or, preferably, at a lower concentration than the normal range, wherein the normal range is the range of concentrations at which the chemotherapeutic agent is usually administered in the absence of other therapeutic agents.

It is anticipated that in embodiments of the invention the binding members and chemotherapeutic agent could be given in combination with other forms of chemotherapy or indeed radiotherapy.

Thus, in a further aspect of the invention, there is provided a method of killing p 53 mutant cancer cells comprising administration of a therapeutically effective amount of a) a specific binding member which binds to a cell death receptor or a nucleic acid encoding said binding member, (b) a chemotherapeutic agent, wherein the chemotherapeutic

HCT116 p53 wild-type and null cell lines following treatment with 5-fluorouracil (5-FU), CPT-11 and Oxaliplatin for 24 and 48 hours. Gene expression was calculated at each timepoint as a ratio of the target gene Fas to 18S. The expression of each gene was calculated according to standard curves generated for each gene using a dilution series. B, Western blot analysis of Fas and p53 expression in the HCT116 p53 wild-type and null cell lines following treatment with 5-FU 5 μ M, CPT-11 5 μ M and Oxaliplatin 1 μ M for 24 hours.

Figure 8 illustrates graphs of RI values calculated from MTT cell viability assays of the chemotherapeutic agents 5-FU, Tomudex (TDX), CPT-11 and Oxaliplatin used in combination with the agonistic anti-Fas antibody CH-11 (200ng/ml). The RI is calculated as ratio of the expected cell survival (S_{exp} , defined as the product of the survival observed with drug A alone and the survival observed with drug B alone) to the observed cell survival (S_{obs}) for the combination of A and B ($RI = S_{exp} / S_{obs}$). Synergism is defined as an RI greater than 1.

Figure 9 illustrates A, Flow cytometry analysis of cells stained with propidium iodide stained HCT116 p53 wild-type and null cells treated with 5-FU (5 μ M), TDX (50nM), CPT-11 (5 μ M) and Oxaliplatin (1 μ M) for 24 hours and then with CH-11 (50ng/ml) for an additional 24 hours. B, Sub G0/G1 populations for the HCT116p53 wild-type and null cell lines treated

cell lines treated with IC60_{72hrs} doses of 5-FU, TDX, CPT-11 and Oxaliplatin for 24 and 48 hours showed significant induction of Fas mRNA expression in response to these agents in the p53 wild-type cells (Fig. 7A). The fold induction of Fas mRNA seen at 24 and 48 hours respectively were 3.8 and 3.4 for 5-FU, 7.0 and 2.5 for CPT-11, and 5.8 and 4.7 for Oxaliplatin. In the HCT116 p53 null cell line treated under similar conditions there was significantly less induction seen, with maximum induction of 2- and 1.9-fold for 5-FU and CPT-11 respectively and no induction seen with Oxaliplatin (Fig. 7A). These results indicate that induction of Fas/CD95 mRNA by these chemotherapeutic agents is p53-dependent.

Example 7 CPT-11 treatment results in a p53-independent induction of Fas/CD95 protein in the HCT116 p53 null cell line

Given that each of the chemotherapeutic agents we examined induced Fas mRNA expression in the HCT116 p53 wild-type cell line following treatment, we analysed whether this was reflected as induction of protein expression. Treatment of the HCT116 p53 wild-type and null cell lines with 5-FU 5 μ M, CPT-11 5 μ M and Oxaliplatin 1 μ M for 48 hours resulted in significant induction of Fas/CD95 by all three chemotherapy drugs in the p53 wild-type cell line. The observed induction in this cell line was associated with induction of p53. In contrast, only CPT-11 treatment in the p53 null cell line resulted

p53 wild-type cell line compared to the p53 null cell line. The magnitude of induction of the Fas/CD95 receptor is much higher than would have been predicted from Western blot analysis (Fig. 7B). The ability of TDX and CPT-11 to interact synergistically with the anti-Fas antibody in the p53 null cell line was associated with induction of the Fas/CD95 receptor by flow cytometry following treatment with these agents for 24 hours. Both 5-FU and Oxaliplatin were only able to significantly induce expression of the receptor in the HCT116 p53 wild-type cell line (Fig. 11 A, B).

Example 11 Fas/CD95 cell surface expression in the p53 mutant H630 and p53 wild-type RKO cell lines following treatment with the chemotherapeutic agents 5-FU, TDX, CPT-11 and Oxaliplatin

Induction of the Fas receptor in the p53 mutant H630 cell line was only seen with CPT-11. Neither 5-FU nor Oxaliplatin treatment for 48 hours caused significant upregulation of the receptor (Fig. 12A). In the p53 wild-type RKO cell line there was significant induction of the Fas receptor in response to IC50 doses of all three chemotherapeutic agents (Fig. 12A). When each of the cell lines was treated with these chemotherapy drugs for 24 hours followed by the anti-Fas antibody CH-11 for an additional 48 hours significant synergy was evident with the CPT-11 combination in the H630 cell line (Fig. 12B). When the RKO cell line was treated under

activation in response to treatment with 5-FU and the antifolates suggests that Fas-mediated apoptosis may be inhibited in MCF-7, HCT116 and RKO cancer cells. However, co-treatment with CH-11 was sufficient to overcome this resistance and activate Fas-mediated apoptosis.

The inventors' findings raise the possibility of using antimetabolite drugs in combination with anti-Fas antibodies as a novel anticancer strategy. Targeting Fas may be particularly useful against tumour cells that overexpress FasL and Fas pathway inhibitors, and which thereby evade Fas-mediated elimination by immune cells. However, systemic treatment with Fas antibodies or rFasL in mouse models has been shown to cause severe damage to liver and other organs (31). Some recent studies have focussed on local administration of rFasL, or the use of FasL-expressing vectors as gene therapy to overcome systemic toxicity (31). In addition, a novel agonistic Fas-targeted antibody HFE7A has been developed recently that was not hepatotoxic in murine models, suggesting that it may be possible to develop less toxic Fas-targeted antibodies (32).

Treatment with TS inhibitors has been shown to acutely induce TS expression in cell lines and tumours (18, 33). Furthermore, pre-clinical and clinical studies have found that TS is a key determinant of sensitivity to 5-FU, with high TS expression correlating with increased resistance (1, 34). The inventors therefore examined the effect of

prostate carcinoma cells. *Cancer Res*, 2003.

63(11): p. 2905-12.

51. He, M., et al., A mutant P53 can activate apoptosis through a mechanism distinct from those induced by wild type P53. *FEBS Lett*, 2002. 517(1-3): p. 151-4.
52. Pugacheva, E.N., et al., Novel gain of function activity of p53 mutants: activation of the dUTPase gene expression leading to resistance to 5-fluorouracil. *Oncogene*, 2002. 21(30): p. 4595-600.
53. Forrester, K., et al., Effects of p53 mutants on wild-type p53-mediated transactivation are cell type dependent. *Oncogene*, 1995. 10(11): p. 2103-11.